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Growth hormone prevents steroid-induced growth depression in health and uremia

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Growth hormone prevents steroid-induced growth depression in health and uremia. Treatment with supraphysiological doses of corticosteroids results in protein wasting and impairment of growth, whereas exogenous growth hormone (GH) causes anabolism and improvement of growth. We wanted to know whether the growth depressing effects of methylprednisolone (MP) are more expressed in an organism which is chronically diseased and whether these effects can be counterbalanced by concomitant treatment with recombinant human growth hormone (rhGH). MP in doses from 1 to 9 mg/kg/day caused a dose dependent reduction of length gain, weight gain and weight gain/food intake ratio in 140 g healthy female Sprague-Dawley rats. Food intake was not affected by MP. This points to a change in food metabolism as a mechanism for growth impairment. In addition, treatment with MP inhibited endogenous GH secretion, documented by serum GH concentration profiles over seven hours, decreased IGF-1 serum concentration and disturbed growth cartilage plate architecture. Concomitant treatment with 2.5 to 20 IU/rhGH/kg/day prevented the negative effects of MP on growth in a dose dependent manner and normallized growth plate architecture. In uremic rats in which food efficiency and growth was already reduced, 6 mg MP/kg/day further decreased length gain and prevented weight gain completely by bringing the weight gain/food conversion ratio to the nadir. All effects of MP including reduction of muscle mass could be prevented by concomitant treatment with 10 IU rhGH/kg/day. The effects of MP and rhGH on food efficiency and growth in uremic animals were numerically nearly identical to those in pair fed and ad libitum fed controls, but this may be more relevant in the diseased organism in which basal growth is already suppressed.

Patients receiving corticosteroid treatment for a prolonged period of time, such as allograft recipients, patients with steroid-dependent nephrotic syndrome, those with autoimmune and asthmatic diseases, and many others, manifest side effects of such treatment. Despite the absence of a marked Cushing syndrome, protein catabolism [1] and growth impairment [2] may result. Clinical observations suggest that patients with an underlying chronic disease are more prone to catabolism and growth failure consequent to corticosteroid treatment than are otherwise healthy individuals. Therefore, it is of paramount importance to identify those factors leading to corticosteroid-induced protein losses and to develop preventative therapeutic strategies.

Recombinant human growth hormone (rhGH) is a potent anabolic agent known to stimulate linear growth in GH deficient children [3] and in the stunted organism without primary GH deficiency like the Turner syndrome [4] and chronic renal failure (CRF) [5–7]. Since rhGH is now available in virtually unlimited amounts, it has been possible to demonstrate that rhGH improves nitrogen balance in healthy volunteers [8], in atheletes [9], in patients receiving parenteral nutrition [10] and in hypocaloric situations [11]. The purpose of the present study was to determine if the growth depressing effects of methylprednisolone (MP) can be counterbalanced by concomitant administration of rhGH and if so, whether such an effect is dose dependent. Furthermore, we addressed the issue of whether a chronic disease would enhance the growth depressing effects of corticosteroids, thereby minimizing the growth promoting effects of rhGH.

Chronic renal failure (CRF) was selected as an example of a chronic disease. Children with CRF are catabolic and grow subnormally [12]. Successful renal transplantation does not normalize growth in a great proportion of patients. The reason is not fully understood [13]; reduced renal function and corticosteroid treatment are two important factors influencing growth negatively. To mimick the transplant situation and to evaluate our hypothesis, an animal model was established to determine the effects of concomitant MP and rhGH treatment.

Methods

Female Sprague-Dawley rats (Ivanovas Co., Kisslegg/Allgäu, Germany), weighing 100 g, were used for the experiment. One week prior to the study, the animals were kept in single cages at constant room temperature (24°C) and humidity (70%) on a 12 hours on/12 hours off light cycle. The diet contained: 13800 kJ/kg; 0.95% calcium; 0.8% phosphorus; 500 IU/kg vitamin D_3; and 18% protein (wt/wt). The animals were subjected to a two-stage subtotal nephrectomy (NX) as described previously [5, 14]. One week after total nephrectomy of the left kidney, the contralateral kidney was removed and uremia resulted when the animals had a mean body weight of 136 g. Control animals (CO) were sham operated (renal decapsulation). Uremic animals and controls had free access to food (Altromin C 1000 diet, Altromin

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Co., Lage, Lippe, Germany) and deionized water. In addition, the control group was pair-fed as previously described [14].

Dose response experiments

For dose response experiments, 125 to 150 g healthy female Sprague-Dawley animals were used. All animals were allowed free access to food and water. (a) For determining the maximal dose of rhGH, groups of six animals each were injected with 0, 2.5, 5.0, 10.0, and 20.0 IU/kg rhGH daily during a period of seven days. (b) For determining the maximal growth depressing effect of MP, six animals per group were injected s.c. with 1, 3, 6, and 12 mg/kg/day during a period of seven days. In addition, submaximal doses of (c) MP (2 mg/kg/day) and of (d) rhGH (2.5 IU/kg/day) were combined with increasing doses of rhGH (0 to 10.0 IU/day) or with increasing doses of MP (0 to 12 mg/kg/day). Experiments a through d were performed at different times.

Experimental protocol for concomitant treatment with MP and rhGH

The animals were randomized into three groups: Group A received solvent twice daily s.c.; Group B received MP 6 mg/kg daily s.c.; Group C received MP 6 mg/kg daily s.c. and rhGH 5 IU/kg twice daily s.c. All groups (A, B, C) were additionally divided into three subgroups of five animals each: uremic animals (NX), sham-operated pair-fed control animals (PF-CO) and sham-operated ad libitum-fed control animals (ad lib-CO). The duration of the experiment was 14 days.

Measurements

All measurements of body weight were performed in non-fasted animals in the afternoon. Nose-tail tip distances were measured in ether anaesthesia and under conditions of deep muscle relaxation as described previously [5, 14]. All organs were weighed before and after desiccation (24 hr; 80°C in the presence of desiccant). Blood was obtained by aortic puncture from non-fasted animals at the end of the experiment. Serum biochemical constituents were measured with a multichannel Technicon Autoanalyzer (Technicon Instruments, Tarrytown, New York, USA). Creatinine was determined by a kinetic method according to Jaffé without deproteinization; the coefficient of variation within assay was <3%. Blood pressure was measured by tail plethysmography as described previously [14].

Rat GH was determined using an RIA technique. Antibodies against rGH and a standard preparation of rGH (RP-2) were supplied by National Hormone and Pituitary Program (Bethesda, Maryland, USA). Rabbit anti-monkey serum was prepared at Kabi (Stockholm, Sweden), and rGH for labelling was donated by Professor Paul Roos, Uppsala, Sweden. Incubation was performed at +4°C, initially with samples (in duplicate; intra assay CV_W 2 to 8% for GH levels between 0.5 to 8 ng/ml) or standards together with primary antibodies overnight, followed by incubation with labelled rGH for a further 16 hours. The complexes were precipitated by second antibodies, and centrifuged. The pellets were counted in a LKB-Wallac Gammacounter. Concentrations were calculated. The standard curve ranged from 0.25 to 16 ng/ml.

For GH pulse analysis (see serum growth hormone profiles below), a PC version of the PULSAR peak analysis program [15] was used. The smoothing window was set to 400 minutes, corresponding to the duration of the profile. A quadratic assay of SD function was employed which was derived from precision data of the GH assay given above. G values of 3.0 (G1), 2.4 (G2), 1.5 (G3), 1.2 (G4) and 1.0 (G5) were employed. In addition, each profile was inspected visually by estimation of multiple CVs to check for the plausability of the computer analysis.

Measurements of IGF-I were done by using an RIA technique. The antibody was provided by Professor Peter Gluckman, New Zealand, and the standard preparation was recombinant hIGF-I (rhIGF-I; Kabi, Stockholm, Sweden). ^{125}I-Iodinated rhIGF-I was used as tracer. Samples were run in duplicate at two or more dilutions. Incubation was performed at +4°C overnight in a total volume of 3 ml. Precipitation was done by the addition of a second antibody in a polyethylene glycol (PEG) solution, and centrifuged. The pellets were counted in a LKB-Wallac Gammacounter. Concentrations were calculated. The standard curve ranged from 0.7 to 11.2 ng/ml.

For histological measurements in the proximal tibia growth zone, the fluorescent marker calcein was administered to animals in a single injection (10 mg, s.c.) one week prior to sacrifice [16]. The proximal tibiae were cut in frontal slices and fixed in 40% (vol/vol) ethanol at +4°C for 24 hours. After dehydration in a graded series of ethanol at +20°C, tissue slices were infiltrated and embedded in methylmethacrylate. After completion of polymerization at 30°C, 5 m thick sections were cut in the femoral plane on a Jung microtome No. 1140. Sections were subsequently mounted on gelatin-coated slides and stained initially by the von Kossa reaction and subsequently with McNeil Tetrachrome [17]. They were used for morphometric estimations of growth plate height. Ten micrometer sections from each tibia were mounted unstained for measurements of growth rates using incident light fluorescence microscopy [18].

Serum growth hormone profiles

For growth hormone (GH) profile studies, a separate set of animals underwent double catheter implantation, according to the method previously described [19]. At the time of the second operation for subtotal nephrectomy or sham operation, silastic cannulas were inserted into the aorta and vena cava through the ventral tail artery and left external iliac vein, respectively. The femoral catheter was tunnelled subcutaneously to the tail root incision, where after wound suture both catheters were exteriorized and fixed by means of an acrylic cuff. The GH studies were performed one week after inducing uremia.

A constant exchange transfusion technique, adapted from Urbanski, Urbanski and Ojeda [20], was used for the GH secretion studies: 10 minutes before the start of the study the catheters were unplugged, flushed with sodium heparin, extended using polyethylene tubing (PE 50), and connected to the sampling apparatus through a flexible steel spiral attached to the plastic cuff. Thus, the animals could move unrestrained within the cage during the study. Both catheters were connected to a peristaltic pump (Minipuls 2, Gilson Medical Electronics, Middleton, Wisconsin, USA), which provided balanced continuous withdrawal of blood through the arterial catheter and infusion of a warmed (37°C) blood replacement mixture through the venous line. Blood was exchanged at a rate of 50 μl/min over a period of 360 minutes. The withdrawn blood was dispensed at 10

Table 1. Effects of methylprednisolone (MP) and recombinant human growth hormone (rhGH) on growth

	Daily treatment		Cumulative weight gain	Cumulative length gain	Cumulative food intake	Weight gain / Food intake
	MP *mg/kg/day*	rhGH *IU/kg/day*	*g*	*cm*	*g*	*g/g*
Experiment a	0	0	23 ± 1.2	2.2 ± 0.12	100 ± 3.3	0.24 ± 0.004
	1	0	20 ± 1.2 [b]	2.0 ± 0.08 [a]	96 ± 1.2	0.21 ± 0.008 [b]
	3	0	18 ± 2.5	2.0 ± 0.12	99 ± 2.0	0.18 ± 0.020
	6	0	14 ± 1.6	1.6 ± 0.12	91 ± 2.5	0.15 ± 0.012
	9	0	14 ± 1.2	1.8 ± 0.08	92 ± 1.6	0.14 ± 0.012
Experiment b	0	0	16 ± 2.9	1.9 ± 0.12	91 ± 4.1	0.17 ± 0.029
	0	2.5	32 ± 2.0 [b]	2.3 ± 0.12 [b]	97 ± 5.3	0.33 ± 0.012
	0	5.0	36 ± 2.5	2.5 ± 0.04	98 ± 3.3	0.36 ± 0.016 [b]
	0	10.0	35 ± 2.5	2.6 ± 0.16	96 ± 4.5	0.36 ± 0.020
	0	20.0	41 ± 2.5	2.6 ± 0.12	106 ± 3.7	0.39 ± 0.020
Experiment c	0	0	20 ± 0.4 [b]	2.1 ± 0.04 [a]	83 ± 2.0	0.24 ± 0.002 [a]
	2	0	9 ± 2.9 [b]	1.5 ± 0.08 [a]	82 ± 3.7 [a]	0.11 ± 0.032 [b]
	2	2.5	21 ± 2.5	1.9 ± 0.12	86 ± 3.7	0.25 ± 0.020
	2	5.0	23 ± 2.5 [b]	2.1 ± 0.16 [b]	91 ± 4.1	0.25 ± 0.020 [b]
	2	10.0	31 ± 2.0	2.4 ± 0.08	94 ± 2.0	0.33 ± 0.020
Experiment d	0	0	16 ± 1.6 [a]	1.8 ± 0.08 [a]	111 ± 3.7	0.15 ± 0.012 [a]
	0	2.5	35 ± 2.5 [b]	2.3 ± 0.08 [a]	115 ± 5.7	0.31 ± 0.020 [a]
	1	2.5	21 ± 2.5 [b]	1.9 ± 0.12 [b]	113 ± 4.5 [a]	0.19 ± 0.016 [b]
	3	2.5	14 ± 2.0	1.5 ± 0.08	108 ± 4.5	0.14 ± 0.016
	6	2.5	19 ± 1.2	1.6 ± 0.04	108 ± 3.7	0.17 ± 0.012
	12	2.5	14 ± 2.0	1.4 ± 0.12	89 ± 3.3	0.16 ± 0.002

Dose Dependency Experiments (n = 6 animals per group, duration of experiments 7 days)
[a] $P < 0.05$
[b] $P < 0.01$

minute fractions and centrifuged approximately every 60 minutes. The plasma was stored frozen until assayed for GH and IGF-I.

The blood replacement mixture was prepared according to the method of Ellis and Desjardins [21]. Red cells from female donor rats were washed several times in physiological saline and resuspended in a charcoal-filtered 2.5% human albumin solution (Behringwerke AG, Marburg, Germany), adjusted to a hematocrit of 40%, and containing 0.5 mg glucose/ml, 2 IE sodium heparin/ml and 100 IU penicillin/ml. The suspension was stored at +4°C for a maximal period of 24 hours prior to use. At most, only traces of steroid or GH were detectable in the substitution fluid.

Statistics

Data are given as X ± SEM and as median and range. Results were evaluated using Wilcoxon's test for random samples or paired differences and the Kruskall Wallis test. Differences at the 5% level were taken as significant.

Results

Dose response experiments

As shown in Table 1, maximal growth stimulating effects for length gain and weight gain were obtained by injecting 10 IU/kg rhGH in two divided doses s.c. daily (Experiment b). The anabolic effect of rhGH is best seen from the food conversion ratio, e.g. weight gain per food intake, which is doubled by a treatment with 5 IU/day (Experiment b). In contrast, MP had a growth depressing effect on length gain and weight gain. Maximal effects were obtained by 6 mg/kg/day, the food conversion ratio was reduced by one third (Experiment a). Results from Experiments c and d demonstrate that the growth stimulating effects of rhGH can be compensated for by MP and vice versa in a dose dependent manner

Table 2. Serum growth hormone profile studies in rats treated with methylprednisolone (MP)

	Pair fed control		Uremia	
	Solvent	MP	Solvent	MP
Mean levels *ng/ml*	8.9 (6.3–27.2)	6.5 (2.2–8.9)	9.6 (5.3–30.6)	6.3 (4.0–10.1)
Total area *min × ng/ml*	3652 (2505–11205)	1831 (885–2197)	5987 (2122–12615)	2882 (1650–3832)
Number of peaks	3 (2–5)	4 (3–6)	4 (1–5)	3 (3–4)
Peak height *ng/ml*	10.1 (6.2–78.5)	6.4 (1.0–8.2)	14.3 (6.6–43.6)	5.6 (2.9–12.6)
Peak area *min × ng/ml*	409 (127–2609)	112 (35–161)	221 (122–1477)	151 (56–224)

Analysis was performed every 10 minutes over a period of 7 hours

Hormonal studies

Since MP may suppress GH secretion, serum GH concentrations were measured every 10 minutes over a period of seven hours in five uremic and five pair fed control animals, treated either with solvent or with 6 mg MP/kg/day (Table 2). There was no difference between control and uremic animals, but the mean GH concentration as well as the total area under the curve were substantially lower in the MP treated animals of both groups. These differences reached significance ($P < 0.05$) when MP animals (control + uremic) were compared with solvent animals of both groups. The number of GH peaks was not influenced by

Table 3. Growth in healthy and uremic rats treated with methylprednisolone (MP) and recombinant human growth hormone (rhGH) s.c.

	Uremia			Pair fed control			Ad libitum fed control		
	Solvent	MP	MP + GH	Solvent	MP	MP + GH	Solvent	MP	MP + GH
N = 10 animals per group									
Wilcoxon test									
Cumulative food intake *g*	163 ± 3[c]	153 ± 14	181 ± 6	163 ± 3	153 ± 4	181 ± 6	193 ± 5	175 ± 5	198 ± 6
Length gain *cm*	2.6 ± 0.15[c]	1.4 ± 0.15[a]	2.4 ± 0.15	3.0 ± 0.18	2.1 ± 0.21[a]	3.2 ± 0.15	3.9 ± 0.11	2.5 ± 0.11[a]	4.0 ± 0.15
Weight gain *g*	25.3 ± 2.3[c]	0.6 ± 1.8[a]	27.2 ± 2.7	29 ± 2.6	3.4 ± 2.2[a]	27.7 ± 1.6	43.5 ± 1.4	20.7 ± 1.7[a]	48.5 ± 1.5
$\frac{\text{Weight gain}}{\text{food intake}} \times 10^{-3}$	0.15 ± 0.02[c]	−0.01 ± 0.01[a]	0.15 ± 0.02	0.18 ± 0.02	0.02 ± 0.02	0.15 ± 0.01	0.23 ± 0.01	0.12 ± 0.01	0.25 ± 0.01
N = 5 animals per group									
Kruskall Wallis test									
Initial length *mm*	352 (342–356)	352 (345–358)	354 (345–375)	361 (352–376)	368 (352–372)	362 (354–380)	358 (332–365)	355 (351–365)	360 (354–365)
Final length *mm*	376 (359–379)	360 (363–360)	378 (368–399)	386 (376–398)	378 (366–384)	386 (376–405)	386 (358–394)	372 (363–390)	388 (378–404)
Initial weight *g*	136 (127–144)	137 (136–145)	141 (136–149)	151 (143–153)	147 (139–158)	145 (140–160)	146 (139–153)	143 (136–166)	144 (143–155)
Final weight *g*	161 (140–166)	136 (135–146)	164 (154–180)	168 (156–177)	151 (149–158)	175 (170–187)	185 (167–191)	156 (143–199)	195 (176–211)
Muscle weight dry *mg* (triceps surae)	298[d] (254–309)	234[b] (217–242)	289 (244–332)	302 (249–341)	256 (215–311)	307 (233–323)	328 (276–366)	296 (283–362)	349 (265–391)
Liver weight dry *mg*	1840 (1700–1950)	1561[b] (1488–1650)	1770 (1468–2085)	1906 (1850–2290)	2319 (2057–2558)	2392 (2094–2864)	2060 (1841–2369)	2167 (1693–2688)	2194 (2163–2740)
Heart weight dry *mg*	96[d] (90–102)	93[b] (88–99)	107 (93–119)	137 (130–161)	136 (134–156)	164 (157–192)	153 (110–178)	138 (124–176)	178 (155–194)
Blood pressure *mm Hg*	147[e] (140–170)	145 (140–165)	147 (130–155)	110 (85–120)	120 (115–130)	120 (115–130)	120 (95–130)	110 (95–130)	115 (95–130)

[a] Difference MP vs. Solvent and MP-GH, $P < 0.01$
[b] Difference MP vs. Solvent and MP-GH, P 0.05
[c] Difference Uremia Solvent vs. Control ad lib, $P < 0.01$
[d] Difference Uremia Solvent vs. Controls solvent, P 0.05
[e] Difference Uremia Solvent vs. Controls solvent P 0.01

MP treatment, but the peak amplitude as well as the mean peak area were significantly ($P < 0.05$) diminished by MP (all MP animals vs. all solvent animals).

The IGF-I serum concentration was measured in controls fed ad lib one week after treatment with MP with increasing doses. There was a significant decrease in the IGF-I concentration: solvent, 615 ± 38 ng/ml; 3 mg MP/kg/day, 495 ± 38 ng/ml; 6 mg MP/kg/day, 524 ± 22 ng/ml; 9 mg MP/kg/day, 390 ± 48 ng/ml (solvent vs. 9 mg MP, $P = 0.02$).

Concomitant treatment with MP and rhGH

The main experiment (Tables 3 to 5) was done with five animals per group. To allow unequivocal statistical analysis, part of the experiment (growth data) was repeated in a second set with ten animals per group (Table 3, upper part). Maximally effective doses of MP and rhGH were given concomitantly to healthy and uremic animals, sham operated control animals were either pairfed or fed ad libitum. Differences in initial weight and length between uremic and control animals were due to the pair feeding protocol [14]. Growth of solvent treated uremic animals was significantly less than in ad libitum fed controls, but only insignificantly different from pair fed animals. In ad libitum fed control animals, MP depressed mean weight gain by more than 50% and length gain by about one third. Concomitant treatment with rhGH prevented the suppression of weight gain and length gain. In uremia, weight gain was suppressed completely, but all effects of MP on growth of uremic animals were numerically within the range of control animals.

Neither MP nor rhGH significantly changed the cumulative food intake of uremic or control animals, although this parameter tended to be reduced under the influence of MP and to be enhanced by rhGH (Table 3). In contrast, MP decreased and concomitant rhGH normalized the food conversion ratio. Again, in uremic animals, the effects of MP and of rhGH were numerically nearly identical when compared to the effects in controls pairfed or fed ad libitum.

Neither MP nor rhGH had a significant effect on blood pressure. In comparison to control animals, blood pressure was significantly higher in uremic animals irrespective of the treatment.

Serum creatinine and urea concentrations were comparable for uremic animals irrespective of treatment with MP and rhGH (Table 4), but were significantly higher when compared to control animals. MP and rhGH had no significant effects on serum creatinine, urea, electrolyte, glucose or protein concentrations in either uremic or control animals. In addition, no significant changes in urinary calcium and urea excretion were noted. Creatinine clearance was not influenced by MP, but was significantly increased by rhGH in the control animals. This effect was not noted in the uremic animals.

The histomorphometric measurements of the tibial metaphysis (Table 5, Fig. 1) confirm that MP significantly suppresses the daily growth rate, which can be reversed by concomitant rhGH

Table 4. Biochemical data in healthy and uremic rats treated with methylprednisolone (MP) and recombinant human growth hormone (rhGH) s.c.

	Uremia			Pair fed control			Ad libitum fed control		
	Solvent	MP	MP + GH	Solvent	MP	MP + GH	Solvent	MP	MP + GH
Serum creatinine *mg/dl*	0.7[b]	0.7	0.8	0.3	0.3	0.3	0.3	0.3	0.3
	(0.6–0.9)	(0.7–0.7)	(0.5–0.8)	(0.2–0.3)	(0.2–0.3)	(0.3–0.3)	(0.2–0.3)	(0.3–0.4)	(0.3–0.3)
Urea *mg/dl*	98[b]	104	102	24	40	31	30	32	27
	(75–142)	(98–122)	(94–142)	(20–28)	(24–41)	(29–38)	(25–37)	(26–36)	(22–29)
K^+ *mmol/liter*	3.9	4.6	4.5	4.0	4.3	4.0	4.1	4.2	4.2
	(3.6–4.2)	(3.7–4.7)	(3.6–4.7)	(3.6–4.2)	(4.3–4.4)	(3.9–4.3)	(3.9–4.5)	(3.5–4.6)	(4.1–4.3)
Ca^{++} *mmol/liter*	2.6	2.6	2.5	2.6	2.5	2.6	2.5	2.5	2.4
	(2.6–2.8)	(2.4–2.7)	(2.4–2.6)	(2.4–2.6)	(2.4–2.6)	(2.5–2.7)	(2.4–2.7)	(2.4–2.6)	(2.4–2.6)
Pi^{++} *mmol/liter*	2.5	2.5	2.6	1.8	2.3	2.3	2.3	2.3	1.8
	(2.1–3.0)	(2.1–3.2)	(2.0–3.1)	(1.7–2.3)	(2.0–2.6)	(2.0–2.5)	(2.2–2.5)	(1.8–2.4)	(1.6–2.1)
Glucose *mg/dl*	92	102	98	79	97	96[c]	93	102	101
	(82–99)	(82–108)	(90–107)	(69–103)	(89–106)	(85–115)	(86–105)	(96–115)	(86–113)
GH (human) *ng/ml*	0.07	0.11	10.0[d]	0.08	0.12	15.1[d]	0.12	0.08	10.9[d]
	(0.05–0.09)	(0.05–0.17)	(3.3–15.7)	(0.04–0.12)	(0.10–0.16)	(3.3–19.7)	(0.02–0.17)	(0.06–0.16)	(2.8–16.3)
GH (rat) *ng/ml*	13.0	13.5	2.5[c]	10.0	8.0	1.0[d]	9.0	12.0	1.0[d]
	(3.0–19.0)	(7.0–19.0)	(0.5–11.0)	(8.0–19.0)	(3.0–14.0)	(0.5–4.0)	(3.0–22.0)	(7.0–14.0)	(0.5–2.0)
Urinary Ca *mmol/day*	0.02	0.02	0.04	0.01	0.01	0.02	0.01	0.01	0.02
	(0.02–0.04)	(0.01–0.05)	(0.03–0.08)	(0.01–0.02)	(0.01–0.02)	(0.01–0.04)	(0.01–0.02)	(0.01–0.02)	(0.01–0.02)
Urea *mg/day*	428	419	378	465	4948	387	516	430	461
	(344–483)	(362–453)	(269–516)	(417–656)	(346–632)	(341–601)	(344–606)	(385–469)	(362–658)
C_{Cr} *l/min/100 g*	348[b]	326	344	884	841	1112[c]	834	820	1230[c]
	(234–434)	(233–410)	(228–447)	(698–1019)	(731–1007)	(921–1428)	(708–1033)	(719–1014)	(940–1482)
HCO_3 *mmol/liter*	21.9	19.5[a]	20.7[a]	—	—	—	21.8	22.2	23.2
	(19.0–23.0)	(15.6–20.7)	(14.9–23.0)	—	—	—	(20.0–25.0)	(20.7–24.4)	(21.1–24.2)
Blood pH	7.36	7.25[a]	7.30[a]	—	—	—	7.35	7.41	7.39
	(7.30–7.43)	(7.15–7.39)	(7.10–7.31)	—	—	—	(7.33–7.14)	(7.35–7.46)	(7.36–7.47)

N = 5 animals per group.
Difference between Uremia and Controls: [a] *P* 0.05; [b] *P* 0.01
Difference between MP + GH vs. MP and Solvent: [c] *P* 0.05; [d] *P* 0.01

Table 5. Histomorphometric evaluation of the growth apparatus in the proximal tibia of healthy and uremic rats treated with methylprednisolone (MP) and recombinant human growth hormone (rhGH) s.c.

	Uremia			Pair fed control			Ad libitum fed control		
	Solvent	MP	MP + GH	Solvent	MP	MP + GH	Solvent	MP	MP + GH
Width of growth zone *m*	250	199[a]	263	296	227[b]	268	252	223[b]	296
	(245–291)	(190–246)	(210–317)	(274–337)	(203–238)	(253–285)	(226–267)	(202–261)	(274–337)
Rate of growth *m/day*	101	51[b]	115	112	79[b]	99	127	76[b]	131
	(94–110)	(45–6)	(95–123)	(101–125)	(68–83)	(95–115)	(116–132)	(71–104)	(125–155)

N = 5 per group.
MP vs. solvent and MP + GH: [a] *P* 0.05; [b] *P* 0.01

treatment in uremic and control animals. The height of the growth zone was reduced by MP treatment and significantly increased by rhGH treatment.

Discussion

The present study demonstrates that the growth depressing effects corticosteroids can be prevented by concomitant administration of rhGH in healthy control rats with and without reduced food intake as well as in rats with CRF. The negative effects of MP on weight gain and length gain were dose dependent and could be counterbalanced in a dose dependent manner by concomitant rhGH. Also disturbance of growth plate architecture by MP was normalized by rhGH. This is in contrast to the pessimistic views of Hyams and Carey [22] and Baxter [23] and provides a rationale for controlled studies in stunted children treated by corticosteroids for various reasons.

The animals used in this experiment were post-pubertal. Since epiphyseal closure does not occur during life span of rats, and growth rate is still high at a body weight of 140 g the rat model can be used for growth experiments. Female rats seem to respond better to rhGH than males [5]. In uremic animals, length gain and weight gain did not differ significantly from pairfed controls but was significantly lower than that observed in ad lib feditum control animals. This difference is primarily explained by the diminished food intake and the reduced weight gain/food intake ratio in uremic animals. The latter is evidence for catabolism and/or diminished anabolism in uremic animals. Interestingly, the food efficiency was also impaired in pairfed controls which had a reduced energy and protein intake.

Already therapeutical doses of MP suppressed length gain and weight gain of healthy animals (Table 1). Possible mechanisms by which MP influences growth are suppression of

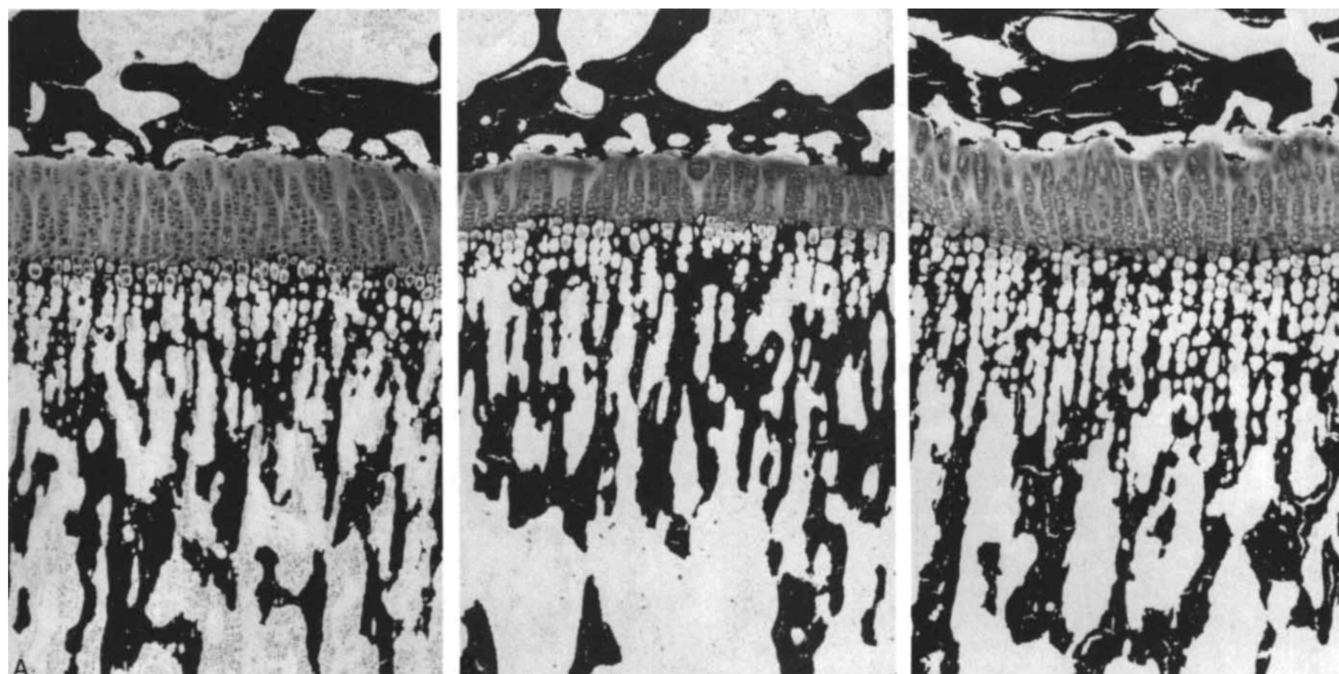

Fig. 1. *Light micrographs of proximal tibia growth plate thick (5µm) sections stained with toluidine blue.* A–C. Sham-operated control animals fed ad libitum receiving either solvent (**A**), methylprednisolone (**B**) or methylprednisolone and growth hormone (**C**). (Figure 1 continued on following page.)

intestinal calcium absorption, protein wasting by catabolism and/or reduced anabolism, inhibition of GH secretion or direct effects on chondrocytes and osteoblasts.

Corticosteroids diminish intestinal calcium absorption [24]. In the present study, MP did not have any effect on urinary calcium and phosphorus secretion, thus confirming the study of Mitchell, Barr and Pocock [25] who demonstrated that corticosteroids have only a minimal effect on calcium absorption in the rat and that calcium supplements do not improve growth.

The weight gain suppression of MP in rats is in contrast to its effect in humans, in whom wasting of muscle mass is compensated for by an increasing fat mass. This increase in fat mass is not seen in rats. The negative effects of MP on linear growth and body weight was paralleled by a negative effect on muscle dry weight. Without changing the food intake MP decreased food conversion ratio in ad libitum fed controls and further impaired it in uremic and pair fed animals in which food efficiency was already reduced. Evidently, inhibition of growth by MP is related to food metabolism and not to food intake. The decrement in weight gain and weight gain/food intake ratio was numerically identical in all groups, but in the uremic and the starving (= pair fed) organism this may be more deleterious, because basal growth is already suppressed.

Controversy exists concerning suppression of endogenous GH secretion by corticosteroids. No or even a stimulatory effect has been reported by various groups [26–28]; however, Pantelakis et al [29] have observed an almost complete disappearance of GH peaks during a 24 hour study. Similarly, we have observed the disappearance of GH peaks in MP treated children following renal transplantation [30]. The discrepancy may be explained by the view that more physiological doses of corticosteroids enhance whereas pharmacological doses suppress growth hormone secretion [31]. The serum GH profiles documented in the present study, support the view that GH secretion is diminished by high doses of MP which may be partly responsible for the growth depressing effect of MP.

If GH secretion is suppressed by MP one should expect low circulating IGF-I levels. Cortisol inhibits the production of IGF-I in liver cells [32] and somatomedin bioactivity was found to be decreased in humans [33] and in rats [34], but also normal serum IGF-I levels have been reported in patients with Cushing syndrome [35] and in subjects receiving corticosteroid therapy [36]. In the present study, peripheral IGF-I concentration was significantly lowered only when 9 mg/kg/day MP was given. This probably indicates a threshold concentration for the effect of MP. However, interpretation of serum IGF-I values in rats requires caution because most RIAs—like ours—are not rat specified and the IGF binding proteins have not been measured concomitantly. Furthermore, peripheral IGF-I may not reflect the local production of IGF-I.

In the present study the number of chondrocytes and the height of the epiphyseal growth plate were reduced by MP. This is consistent with a previous report for rats [37], in which a decrease in the size of chondrocytes and a reduced ability of chondrocytes to replicate and to hypertrophy to osteoblasts were documented. The cellular effects of corticosteroids appear to be dependent on the concentration at the target organs including growth cartilage and bone. In physiological concentrations, corticosteroids positively modulate the responsiveness of cells to anabolic effects of exogenous IGF-I [38] and regulate the basal activity and hormone responsiveness of osteoblasts [39]. They also up-regulate receptors for various hormones, like

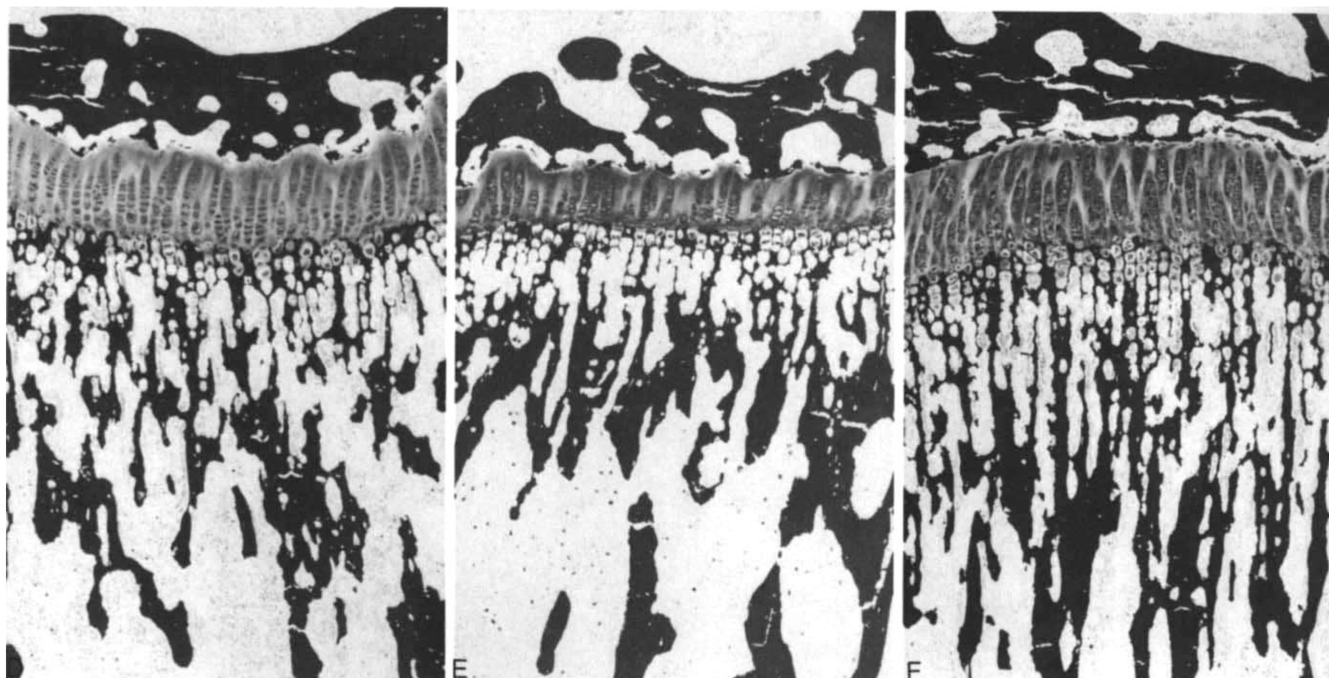

Fig. 1. Cont'd. D–F. Uremic animals receiving solvent (**D**), methylprednisolone (**E**) or methylprednisolone and growth hormone (**F**). In control and uremic animals, height of growth plate is reduced by methylprednisolone (B and E). The reduction in height is attributable to a decrease in the number of proliferating and mainly hypertrophic chondrocytes within cell columns. Concomitant treatment with growth hormone (C and D) not only normalized the height of growth plate but increased the number of hypertrophic chondrocytes in comparison with solvent treated animals (A and D). (× 125: 1).

$1,25(OH)_2D_3$ [40] and PTH [41]. High doses of corticosteroids decrease bone formation [42] and interfere with skeletal growth at the sites of growth cartilage [37]. Potential mechanisms which may explain the negative effects of high dose corticosteroids on growth, are the inhibition of local production of IGF-I [43, 44].

rhGH in supraphysiological doses improves growth in short children with [3] and without GH deficiency [4], and in children and animals with CRF [5–7]. It leads to positive nitrogen balance in stunted children [45], healthy adult volunteers [8] and in hypocaloric situations [11]. For epiphyseal growth, GH acts directly, via peripheral IGF-I and by local production of IGF-I on the proliferation and differentiation of chondrocytes [46]. Taking all studies on the effects of MP and rhGH together one might speculate that the local production of IGF-I and the modulation of cell response to IGF-I are the final common pathway affected by both corticosteroids and GH.

In the present study rhGH counterbalanced the growth depressing effects of MP in a dose dependent manner. In comparison to humans, higher doses of rhGH are needed in rats to see significant effects of rhGH on growth [5]; marked effects were noted with 2.5 IU/kg/day (Table 1) which is more than ten times the dose used in uremic children. The increments in length gain, weight gain and weight gain/food intake ratio were numerically nearly identical in both control groups and in uremic rats. It is tempting to try to compensate the negative effects of corticosteroids on growth by rhGH in clinical studies. Recently, Horber and Haymond demonstrated that N wasting by prednisone can be prevented by concomitant rhGH in humans [8]. We and others could improve growth and muscle mass by rhGH in some, but not all children with renal allografts treated by corticosteoids [47–49]. Remarkably, our patients who did not grow despite normal glomerular filtration rate (GFR) responded well to rhGH. This points to steoids as a major determinant for growth suppression in these children.

Several potential side effects must be considered in such studies. Carbohydrate intolerance and insulin resistance has been noted following administration of either substance [50, 51]. Glomerular hyperfiltration [52] following rhGH treatment must be discussed as a reason for deterioration of renal function in the long run. In this study, C_{Cr} was not affected by MP but increased significantly by rhGH in controls. The increase in C_{Cr} was not paralleled by a change in serum creatinine concentration, which is dependent on renal function and muscle mass and metabolism. Under rhGH treatment muscle is in an anabolic state and it is not known whether steady-state conditions for creatinine have been reached after 14 days. The effect of rhGH on C_{Cr} was abolished in uremic animals. Although GFR was measured by an imperfect method in a small number of animals, the results are in line with our short-term inulin studies in man which showed that C_{In} was increased by rhGH only in healthy controls but not in patients with CRF [53]. The reason for the different renal effects of rhGH in health and CRF may be resistance to GH in uremia or absence of glomerular reserve capacity. Finally, it has to be established whether rhGH counterbalances not only the growth depressing effects of MP but also the immunosuppression. Clinical studies in allograft recipients [47–49] are not conclusive at this moment.

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References

1. Horber FF, Zuercher RM, Herren H, Crivelli MA, Robotti G, Frey FJ: Altered body fat distribution in patients with glucocorticoid treatment and in patients on long-term dialysis. *Am J Clin Nutr* 43:758–769, 1986
2. Friedman M, Strang LB: Effect of long-term corticosteroids and corticotrophin on the growth of children. *Lancet* ii:568–572, 1966
3. Bierich JR: Multicentric clinical trial of authentic recombinant somatropin in growth hormone deficiency. *Acta Paediatr Scand* (suppl) 337:135–140, 1987
4. Rosenfeld RG, Hintz RL, Johanson AJ, et al: Three-year results of a randomized prospective trial of methionyl human growth hormone and oxandrolone in Turner syndrome. *J Pediatr* 113:393–400, 1988
5. Mehls O, Ritz E, Hunziker EB, Eggli P, Heinrich U, Zapf J: Improvement of growth and food utilization by human recombinant growth hormone in uremia. *Kidney Int* 33:45–52, 1988
6. Koch VH, Lippe BM, Nelson PA, Boechat MI, Sherman BM, Fine RN: Accelerated growth after recombinant human growth hormone treatment of children with chronic renal failure. *J Pediatr* 115:365–371, 1989
7. Tönshoff B, Mehls O, Heinrich U, Blum WF, Ranke MB, Schauer A: Growth-stimulating effects of recombinant human growth hormone in children with end-stage renal disease. *J Pediatr* 116:561–566, 1990
8. Horber FF, Haymond MW: Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *J Clin Invest* 86:265–272, 1990
9. Crist DM, Peake GT, Egan PA, Waters DL: Body compositon response to exogenous GH during training in highly conditioned adults. *J Appl Physiol* 65:579–584, 1988
10. Ziegler TR, Young LS, Manson MJ, Wilmore DW: Metabolic effects of recombinant human growth hormone in patients receiving parenteral nutrition. *Ann Surg* 208:6–15, 1988
11. Manson JM, Wilmore DW: Positive nitrogen balance with human growth hormone and hypocaloric intravenous feeding. *Surgery* 100:188–197, 1986
12. Chantler C: Nutritional assessment and management of children with renal insufficiency, in *End-Stage Renal Disease in Children*, edited by Fine RN, Gruskin AB, Philadelphia, WB Saunders, pp. 193
13. Gruskin CM, Fine RN: Growth in children and adolescents following renal transplantation. *Am J Dis Child* 121:514, 1973
14. Mehls O, Ritz E, Gilli G, Schmidt-Gayk H, Krempien B, Kourist B, Wesch H, Prager P: Skeletal changes and growth in experimental uremia. *Nephron* 18:288–300, 1977
15. Merriam GR, Wachter KH: Algorithms for the study of episodic hormone secretion. *Am J Physiol* 243:E310–E318, 1982
16. Schenk R, Eggli P, Fleisch H, Rosini S: Quantitative morphometric evaluation of the inhibitory activity of new aminobisphosphonates on bone resorption in the rat. *Calcif Tis Int* 38:342–349, 1986
17. Cruz-Orive LM, Hunziker EB: Stereology for anisotropic cells: Application to growth cartilage. *J Microsc* 143:47–80, 1986
18. Hunziker EB, Schenk RK, Cruz-Orive LM: Quantitation of chondrocyte performance in epiphyseal plates during longitudinal bone growth. *J Bone Jt Surgery* (Americ Volume) 69A:162–173, 1987
19. Schaefer F, Nedbal U, Mehls O, Przylipiak A, Kiesel L, Schärer K: Uremia effects pulsatile luteinizing hormone secretion in ovariectomized rats. *Neuroendocrinology* (in press)
20. Urbanski HF, Urbanski D, Ojeda SR: An automated system for the study of pulsatile hormone secretion in the immature rat. *Neuroendocrinology* 38:403–406, 1984
21. Ellis GB, Desjardins C: Male rats secrete luteinizing hormone and testosterone episodically. *Endocrinology* 110:1618–1627, 1982
22. Hyams JS, Carey DE: Corticosteroids and growth. *J Pediatr* 113:249–254, 1988
23. Baxter JD: Mechanisms of glucocorticoid inhibition of growth. *Kidney Int* 14:330–333, 1978
24. Kimberg DV, Baerg RD, Gershon E, Grandusius RT: Effect of cortisone treatment on the active transport of calcium by the small intestine. *J Clin Invest* 50:1309–1321, 1971
25. Mitchell I, Barr DGD, Pocock SJ: Catch-up growth in cortisone-treated rats: Effects of calcium and vitamin D. *Pediatr Res* 12:1105–1111, 1978
26. Morris HG, Jorgensen JR, Jenkins SA: Plasma growth hormone concentration in corticosteroid-treated children. *J Clin Invest* 47: 427–435, 1968
27. Roet AW, Bongiovanni AM, Eberlein WR: Studies of the secretion and metabolism effects of human growth hormone in children with glucocorticoid-induced growth retardation. *J Pediatr* 75:826–832, 1969
28. Bridson WE, Kohler PO: Cortisol stimulation of growth hormone production by human pituitary tissue in culture. *J Clin Endocrinol Metab* 30:538–543, 1970
29. Pantelakis SN, Sinaniotis CA, Sbirakis S, Ikkos D, Doxiadis SA: Night and day growth hormone levels during treatment with corticosteroids and corticotrophin. *Arch Dis Child* 47:605–608, 1972
30. Schaefer F, Stanhope R, Preece MA, Schärer K, and the Cooperative Study Group on Pubertal Development in Chronic Renal Failure (CSPDCRF): Pulsatile growth hormone secretion in peripubertal patients with chronic renal failure. *J Pediatr* (in press)
31. Ceda GP, Davis RG, Hoffman AR: Glucocorticoid modulation of growth hormone secretion in vitro. Evidence for a biphasic effect on GH-releasing hormone. *Acta Endocrinol (Copenh)* 114:465–469, 1987
32. Binoux M, Lassarre C, Hardouin N: Somatomedin production by rat liver in organ culture. III. Studies on the release of insulin-like growth factor and its carrier protein measured by radioligand assays. Effects of growth hormone, insulin and cortisol. *Acta Endocrinol (Copenh)* 99:422–430, 1982
33. Phillips LS, Herington AC, Daughaday WH: Hormone effects on somatomedin action and somatomedin generation, in *Advances in Human Growth Hormone Research*, edited by Raiti S, Department of Health, Education and Welfare publication no. (NIH) 74-612, Washington DC, p. 50
34. Mosier HD, Jansons RA, Hill RR, Dearden LC: Cartilage sulfation and serum somatomedin in rats during and after cortisone-induced growth arrest. *Endocrinology* 99:580–585, 1976
35. Thoren M, Hall K, Rähn T: Somatomedin A levels in patients with Chushing's disease. *Acta Endocrinol (Copenh)* 97:12–18, 1981
36. Gourmelen M, Gerard F, Binoux M: Serum somatomedin/insulin-like growth factor (IGF) and IGF carrier levels in patients with Cushing's syndrome or receiving glucocorticoid therapy. *J Clin Endocrinol Metab* 54:885–891, 1982
37. Dearden LC, Mosier HD, Brundage M, Thai C, Jansons R: The effects of different steroids on costal and epiphyseal cartilage of foetal and adult rats. *Calcif Tissue Res* 246:401–412, 1986
38. Kream BE, Petersen DN, Raisz LG: Cortisol enhances the anabolic effects of insulin-like growth factor I on collagen synthesis and procollagen messenger ribonucleic acid levels in cultured 21-day fetal rat calvariae. *Endocrinology* 126:1576–1583, 1990
39. Wong GL: Basal activities and hormone responsiveness of osteoclast-like and osteoblast-like bone cells are regulated by glucocorticoids. *J Biol Chem* 254:6637–6641, 1979
40. Chen TL, Cone CM, Morey-Holton E, Feldman D: 1,25-Dihydroxyvitamin D_3 receptors in cultured rat osteoblast-like cells. Glucocorticoid treatment increases receptor content. *J Biol Chem* 258:4350–4355, 1983

41. YAMAMOTO I, POTTS JT JR, SEGRE GV: Glucocorticoids increase parathyroid hormone receptors in rat osteoblastic osteosarcoma cells (ROS 17/2). *J Bone Miner Res* 3:707–711, 1988
42. CHYUN YS, KREAM BE, RAISZ LG: Cortisol decreases bone formation by inhibiting periosteal cell proliferation. *Endocrinology* 114:477–482, 1984
43. LUO J, MURPHY LJ: Dexamethasone inhibits growth hormone induction of insulin-like growth factor-I (IGF-I) messenger ribonucleic acid (mRNA) in hypophysectomized rats and reduces IGF-I mRNA abundance in the intact rat. *Endocrinology* 125:165–171, 1989
44. McCARTHY TL, CENTRELLA M, CANALIS E: Cortisol inhibits the synthesis of insulin-like growth factor-I in skeletal cells. *Endocrinology* 126:1569–1575, 1990
45. TANNER JM, HUGHES PCR, WHITEHOUSE RH: Comparative rapidity of response of height, limb muscle and limb fat to treatment with human growth hormone in patients with and without growth hormone deficiency. *Acta Endocrinol* 84:681–696, 1977
46. ISAKSSON OGP, LINDAHL A, NILSSON A, ISGAARD J: Mechanisms for the stimulatory effect of growth hormone on longitudinal bone growth. *Endocrin Rev* 8:426–438, 1987
47. JOHANNSSON G, JANSSENS F, PROESMANS W, VANDERSCHUEREN-LODEWEYCKX M, HOLMBERG C, SIPILÄ I, BROYER M, RAPPAPORT R, ALBERTSSON, WIKLAND K, BERG U, JODAL U, RITZEN M, REES L, RIGDEN S, PREECE M, SIETNIKS A: Treatment with Genotropin in short children with chronic renal failure, either before active replacement therapy or with functioning renal transplants. An interim report on five European studies. *Acta Paediatr Scand* (Suppl) 370:36–42, 1990
48. TÖNSHOFF B, TÖNSHOFF C, PINKOWSKI J, HEINRICH U, BLUM WF, RASCHER W, MEHLS O: Effects of recombinant human growth hormone (rhGH) on growth, kidney function and immune system in renal transplanted children. (abstract) *Nephrol Dial Transplant* 5:754, 1990
49. FINE RN, YADIN O, NELSON PA, GRIMM KP, BOECHAT MI, LIPPE BH, SHERMAN BM, ETTENGER RB, KAMIL E: Recombinant human growth hormone treatment of children following renal transplantation. *Pediatr Nephrol* 5:147–151, 1991
50. RIZZA RA, MANDARINO LJ, GERICH JE: Cortisol induced insulin resistance in man. Impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin. *J Clin Endocrinol Metab* 54:131–138, 1982
51. WALKER J, CHAUSSAIN JL, BOUGNÈRES PF: Growth hormone treatment of children with short stature increases insulin secretion but does not impair glucose disposal. *J Clin Endocrinol Metab* 69:253–258, 1989
52. BRENNER MB, MEYER TW, HOSTETTER TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular necrosis in aging, renal ablation and intrinsic renal disease. *N Engl J Med* 307:652–659, 1982
53. HAFFNER D, ZACHAREWICZ S, MEHLS O, HEINRICH U, RITZ E: The acute effect of growth hormone on GFR is obliterated in chronic renal failure. *Clin Nephrol* 32:266–269, 1989